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Biological Characterization of Tomato brown rugose fruit virus-Antalya Isolates

Derya DEMİRALAN, Eminur ELÇİ

Plant Production and Technologies Department, Agricultural Sciences and Technologies Faculty, Niğde Ömer Halisdemir University, Niğde, Türkiye
Correspondence: eminur@gmail.com

Abstract

Tomato brown rugose fruit virus (ToBRFV, genus *Tobamovirus*) is a major pathogen of *Solanaceous* crops in the world. ToBRFV presents a significant and destructive threat to tomato and pepper plants. Türkiye, an important player in the global tomato supply chain, contributes approximately 13 million metric tons annually, ranking it third worldwide in production volume, following China and India. The majority of the production takes place under greenhouse conditions in the Mediterranean Region of Türkiye. Noteworthy, Antalya, Bursa, and Manisa provinces emerged as the foremost tomato-producing regions in 2023. Therefore, the virus inoculum sources used in this study were obtained from Antalya province. The main objective of this study was to investigate ToBRFV symptom characterization in host plants. For this purpose, in 2024, tomato and pepper varieties were mechanically inoculated with ToBRFV, and disease severity was assessed using a standardized disease severity index. In order to establish a control group, mock inoculated plants were used for each variety. The results revealed that some varieties exhibited severe symptoms, while others showed moderate symptoms, and one variety exhibited only mild symptoms. Based on these symptoms, some varieties are considered to be highly susceptible, others are susceptible and one shows tolerance. A key strategy for management of viral diseases is the development and deployment of resistant cultivars, for which disease epidemiology is crucial.

Key Words: *Tobamovirus fructirugosum, tomato, pepper, mechanical inoculation, disease severity index*

Introduction

The cultivated tomato, *Solanum lycopersicum* belongs to the *Solanaceae* family, a taxon of considerable global economic and agricultural significance. Tomatoes represent a substantial dietary source of essential micronutrients, notably vitamins C and E, the carotenoid lycopene (constituting approximately 80% of total carotenoids), folate, and various flavonoids (Willcox et al., 2003). The substantial nutritional value of tomatoes, attributed to their rich vitamin content and inherent antioxidant properties, underscores their status as a globally significant horticultural crop (Viuda-Martos et al., 2014). Specifically, lycopene, a red carotenoid pigment prevalent in tomatoes and processed products like ketchup, has been quantified in the range of 9.9–13.44 mg per 100 g (Story et al., 2013). This carotenoid contributes significantly to the antioxidant capacity and potential health benefits associated with tomato consumption. According to data released by the Food and Agriculture Organization of the United Nations (FAO) in 2023, the annual global tomato production reached approximately 186 million metric tons. Türkiye, a key player in the global tomato supply chain, contributes approximately 13 million metric tons annually, ranking it third worldwide in production volume, following China and India (TÜİK, 2023).

Tomatoes are cultivated in either small or large quantities across numerous regions of Türkiye. The majority of the production takes place under greenhouse conditions in the Mediterranean Region, particularly in Antalya. The other significant tomato-producing regions within Türkiye include the Aegean and Marmara regions. Noteworthy, in 2023, Antalya, Bursa, and Manisa provinces emerged as the foremost tomato-producing regions. Mersin, Çanakkale, Muğla, Tokat, and Çanakkale are significant provinces where tomato production is focused on table tomatoes. A substantial portion of the production in Izmir, Manisa, Balıkesir, and Şanlıurfa is evaluated in the production of tomato sauce.

Tomato cultivation is persistently challenged by a variety of pests and pathogens, among which viruses represent a significant threat to production. Prominent viral pathogens affecting tomatoes include cucumoviruses, tospoviruses, begomoviruses, potyviruses, and tobamoviruses. Among these, the Tobamovirus genus holds particular significance, as it encompasses several highly destructive pathogens such as tomato mosaic virus (ToMV), cucumber green mottle mosaic virus (CGMMV), tobacco mosaic virus (TMV), pepper mild mottle virus (PMMoV), and tomato mild mottle virus (ToMMV). Recently, tomato brown rugose fruit virus= Tobamovirus fructirugosum (ToBRFV) has emerged as a rapidly spreading RNA virus, primarily targeting peppers and tomatoes, with tomatoes being its primary host.

In tomatoes, ToBRFV infection is characterized by mosaic patterns, dark green blistering, yellow mottling, and leaf deformation, and narrowing of the leaves. Affected fruits exhibit yellow blotches, necrotic spotted, and brown rugose or wrinkled patches often resulting in loss of fruit yield (Salem et al., 2023). While tomato and pepper are

recognized as primary natural hosts, ToBRFV exhibits a broader host range. Experimental inoculation has demonstrated symptomatic infection in *N. glutinosa*, *N. tabacum*, *N. benthamiana*, and *N. sylvestris*. Furthermore, the observation of ToBRFV symptoms in weed species, such as *Solanum nigrum* and *Chenopodiastrum murale*, suggests their potential role as viral reservoirs, facilitating the persistence and spread of ToBRFV (EPPO, 2020).
ToBRFV is noted as a seed-borne virus, with contaminated seeds serving as the primary vector for long-distance dissemination. While mechanical contact facilitates short-distance spread, long-distance transmission occurs predominantly through infected seeds and fruits. The agricultural workers' hands, tools, shoes, and clothes can mechanically spread the virus, as well as plant-to-plant contact between infected and healthy plants (Salem et al., 2016). ToBRFV-infected plants exhibited 100% contamination from the seeds of tomatoes harvested. But just externally on the seed coat of tomato, ToBRFV was determined. Transmission appeared at a low rate from seed to seedling from the contaminated seeds. However, no evidence currently supports transmission by arthropod vectors. The Bombus terrestris (bumblebee) beneficial pollinator can carry away the primary inoculum of ToBRFV, thereby promoting the spread of ToBRFV in tomato crops cultivated in glasshouses or net houses (Dombrovsky et al., 2017; Levitzky et al., 2019).
ToBRFV has been listed in quarantine status in the European Union (EU) countries. Since there is no chemical control in viral diseases, the disease has gained even more importance. ToBRFV management is prevention of infection, hygiene, phytosanitary measures, host resistance, disinfection, biocontrol, and gene editing (Salem et al., 2023). Moreover, ToBRFV is one of the most new major virus diseases in tomato plants in our country. Therefore, the main purpose of this study is characterization of symptoms on tomato varieties and pepper.

Materials and Methods

Materials

This study evaluated the performance of five tomato (*Solanum lycopersicum*) varieties, one pepper (*Capsicum annuum*) variety and one wild-type tomato accession *Solanum pimpinellifolium* (LA1651-used as a control). Commercially available tomato varieties included Rio Grande, H2274, and Pintek. The local tomato and pepper cultivars were “Sazlıca tomato” and “Bor pepper”. Pintek and *S. pimpinellifolium* (LA1651) were specifically included due to their reported resistance or tolerance levels to ToBRFV. ToBRFV-infected tomato plant material, used as the inoculum source, was obtained from the T.R. Ministry of Agriculture and Forestry Plant Health Department, Batı Akdeniz Agricultural Research Institute (BATEM). The experiments were conducted within the controlled greenhouse environment and virology laboratory of the Department of Plant Production and Technologies, Niğde, Türkiye.

Methods

Experimental design

Given the highly contagious and deleterious nature of ToBRFV, this research was conducted in the controlled environment of a greenhouse at the Department of Plant Production and Technologies, Niğde Ömer Halisdemir University, Niğde, Türkiye, during the summer of 2024. The recorded highest average temperature was 35°C, while the lowest was 25°C throughout the summer season. The entire process was conducted between May and August. During this period, the average night temperature was 18°C, and the average daytime temperature reached 27°C in the Niğde province.

Seed Germination

In 2024, the tomato and pepper seeds were sown in the seed viols and subsequently, they were allowed to germinate within the same viols. The each viols includes thirty two holes, and a total of seven seed viols were used for each variety. A total of two hundred ten tomato seeds were sown and each variety included thirty seeds and the pots were utilized for transplanting and a total thirty pots were used for each variety.

Seedling Transplanting

Tomato seedlings were transplanted into pots after the germination of seeds. The seed viols were filled with a mixture of perlite and peat in a ratio of 3:1 while 210 plastic pots were filled with soil and a mixture of peat and perlite (3:1), respectively. For each variety, 30 pots were used and pots contained a total of 150 tomato seedlings and 30 Bor pepper seedlings. Also, the were used total of 30 plants as a control. The tomato and pepper seedlings were irrigated before transplantation and the following day, they were transplanted into the pots. The pots were regulated and labelled according to their properties in order to facilitate identification and the tomatoes were irrigated daily.

Mechanical inoculations of tomato and pepper plants

Following the confirmation of ToBRFV infections on tomato and tobacco plants by symptomatic and molecular analyses, fresh tomato leaves were utilized as an inoculum source for mechanical inoculations on both tomatoes and pepper plants. The leaf samples were manually ground with an inoculation buffer (pH: 7.4) consisting of 1.14 g/l Na_2HPO_4, 0.199 g/l KH_2PO_4, 1% PVP-40, and 0.1% Na_2SO_3. The inoculation was conducted on June 2024. Reinoculations were done after 10-14 days. In order to established a control group mock inoculated plants were used for each variety. The symptoms exhibited by tomato and pepper plants were observed 10 days after the mechanical inoculation and symptoms were monitored for 4 weeks for disease severity index evaluation.

Disease Severity Index (DSI) Evaluation

Following a 30-day period after mechanical inoculation, the severity of ToBRFV symptoms was evaluated using a modified disease severity index (DSI). The evaluation of plant symptoms was conducted in accordance with the 0 to 3 scale reported by Zinger et al. (2021). The DSI employed a 0-3 scale to visually assess symptom development in the inoculated plants. Alongside the DSI assessment, the percentage of ToBRFV-infected plants was also determined. DSI values were then calculated using the formulas (Figure 1) (Kabas et al., 2021; Chiang et al., 2017).

$$DSI(\%) = \frac{\text{Class frequency x score of rating class}}{\text{(Total number of observations) x(Maximal disease index)}} \times 100$$

Figure 1 . Disease severity index formulas

Results and Discussion

Virus Symptom Observations on Inoculated Plants

The study includes a total plant of 150 tomatoes and 30 pepper plants inoculated with ToBRFV on May under greenhouse conditions. All inoculated plants showed ToBRFV symptoms and were mostly observed on the leaves of the plant. The onset of symptoms was observed between 10 and 15 days following inoculation and the most common symptoms included mosaic of dark and light green, chlorotic, blistering, narrowing, leaf curling, and mottling observed on tomato and pepper plants.

H2274 and Pintek tomato varieties showed the same symptoms like malformation and deformation of leaves, narrowing, blistering, and very severe mosaic and mottling symptoms on leaves. Sazlıca and Rio Grande tomato varieties showed similar symptoms such as severe mosaic and yellow or green spotting, blistering, and upward curling edges of leaves. Moreover, Bor pepper variety showed symptoms such as mild mosaic and narrowing symptoms of leaves. In addition, the wild type of tomato (*S. pimpinellifolium*) showed symptoms very mild mosaic and mottling symptoms (Figure 2).

ToBRFV -Disease Severity Indices of tested plants

To demonstrate the impact of ToBRFV on tomato and pepper plants, a scale was constituted by ranking the plants from mild to severe (0 to 3). When creating the disease severity scale, the plant exhibiting the least symptoms was assigned a value of 1, while the plant exhibiting the most symptoms was assigned a value of 3. The plant disease value 1 was selected from infected plants with slight chlorosis, and mosaic form on leaves. Plant disease value 2 was selected from infected plants with severe mosaic form and blistering on leaves. Plant disease value 3 was selected from infected plants with very severe leaf narrowing, blistering on the leaf and wilt. This score rate is intraspecific and interspecific of varieties.

Moreover, to evaluated and determined H2274, Pintek, Sazlıca and Rio Grande tomato varieities symptom score value was 3 and Pintek, Sazlıca, Rio Grande, Bor pepper and *S. pimpinellifolium* tomato vaieities symptom score value was 2 also just bor pepper and *S.pimpinellifolium* varieties symptom score value was 1 after the thirty days of inoculation (Figure 3).

Furthermore, the highest infection value was observed in H2274 tomatoes, with a 100% rate, followed by Pintek varieties at 93.3%. In contrast, the wild type of tomato variety (*S.pimpinellifolium*) development the lowest infection rate of with 70% (Table 1).

The objective of this study was to characterize symptoms of ToBRFV on tomato and pepper varieties. Characteristic symptoms were included leaf deformation, mosaic patterns, green or yellow mottling, and leaf narrowing. Symptom onset occurred between 5 to 10 and 15 days post-inoculation. These findings are consistent with those reported by Salem et al. (2023), who observed similar symptoms of leaf mottling, mosaic, and narrowing in tomato plants infected with ToBRFV. Further, in the H2274 and Pintek tomato varieties, we observed symptoms such as leaf malformation, deformation, narrowing, leaf deformation, and severe mosaic and mottling. In the Sazlıca and Rio Grande varieties, severe mosaic, yellow or green spotting, leaf deformation, and upward leaf curling were prominent. Interestingly, milder symptoms, including mild mosaic and leaf narrowing, were observed in the wild-type tomato (*S. pimpinellifolium*) and Bor pepper varieties. Furthermore, Jewehan et al. (2021) reported that they observed very mild mosaic symptoms in *S. pimpinellifolium* tomatoes or no symptoms similar to those in this study.

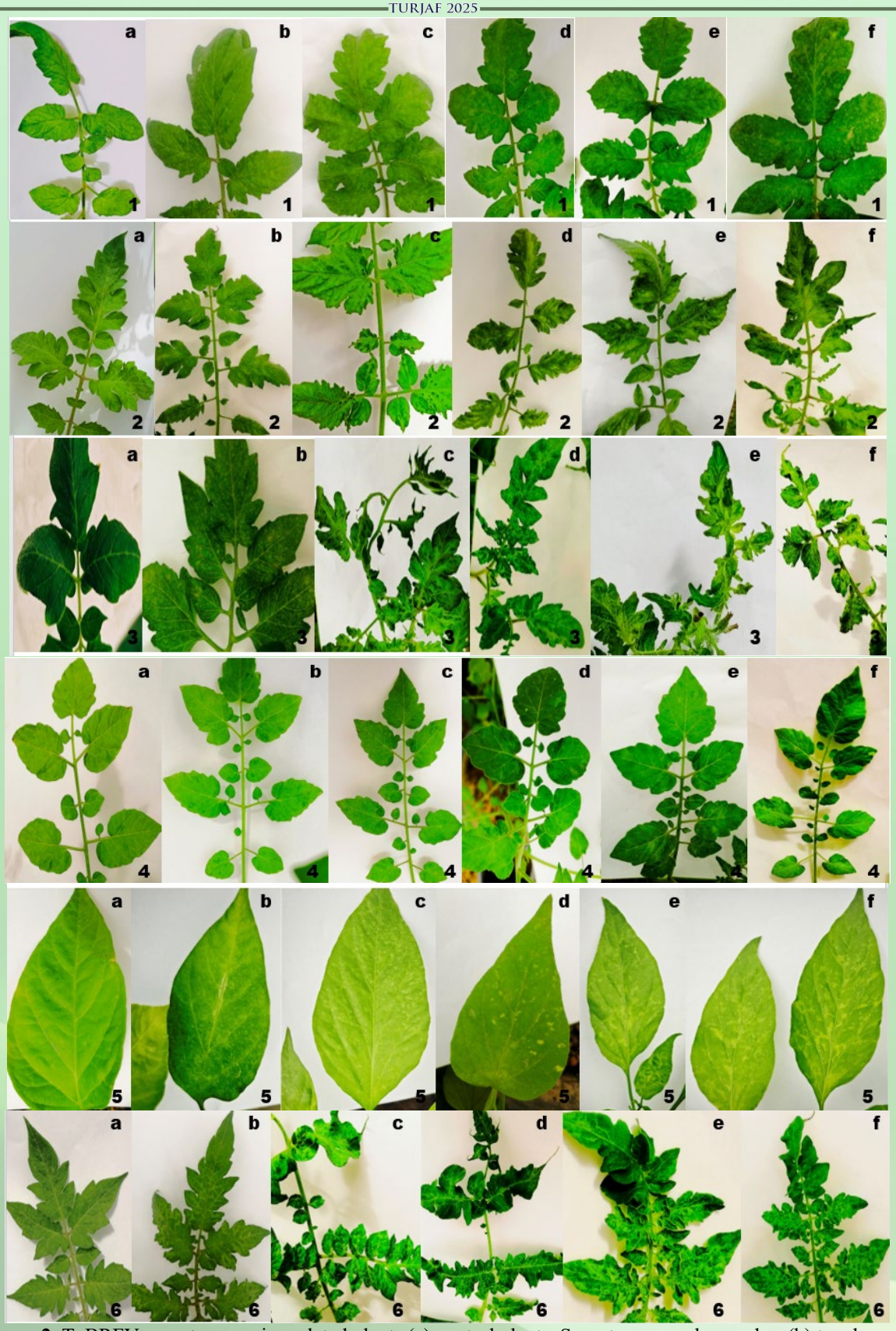

Figure 2. ToBRFV symptoms on inoculated plants (a) control plants, Symptoms are observed as (b) ten days, (c) fifteen days, (d) twenty days, (e) twenty five days and (f) thirty days after the mechanical inoculation. The plant numbers 1 was Rio Grande variety, 2 was Pintek variety, 3 was H2274 variety, 4 was wild type of tomato *S.pimpinellifolium*, 5 was Bor pepper variety and 6 was Sazlıca tomato variety.

Figure 3. Disease severity scales of tested varieties (a) all H2274 tomato score rate is 3, (b) Rio Grande varieties of right pot score rate is 3 and left pot score rate is 2, (c) Pintek varieties of right pot score rate is 3 and left pot score rate is 2, (d) Sazlıca varieties of right pot score rate is 3 and left pot score rate is 2, (e)Bor pepper varieties of right pot score rate is 2 and left pot score rate is 1, (f) wild type of tomato (*S. pimpinellifolium*) varieties of right pot score rate is 2 and left pot score rate is 1.

Additionally, the study focused on disease severity index of ToBRFV infected tomato and pepper plants, including disease infection rate of the plants. To assess the impact of ToBRFV on tomato and pepper plants, disease symptoms were monitored at 10, 15, 20, 25, and 30 days post-mechanical inoculation. A disease severity index was developed to quantify the severity of infection. A scale from 0 to 3 was employed, where 0 represented no

symptoms and 3 indicated the most severe symptoms. Disease severity was assessed at 30 days post-inoculation and the highest infection rate (100%) was observed in H2274 tomatoes, followed by Pintek (93.3%), Bor pepper (85%), and Sazlıca (83%). Rio Grande exhibited a 73% infection rate. In contrast, the wild-type tomato variety *S. pimpinellifolium* (LA1651) displayed the lowest infection rate (70%). These findings align with previous research by Kabas et al. (2022), who reported infection rates ranging from 38% to 60% in two *S. pimpinellifolium* accessions (LA1579 and LA0442).

A comprehensive ToBRFV management plan integrates multiple approaches such as prevention of initial infection, rigorous hygiene practices, utilizing host plant resistance where available, regular disinfection, exploring potential biocontrol agents, and investigating advanced techniques like gene editing for enhanced resistance.

Table 1. Disease severity classes of inoculated plants

Plant	Score number 3 (Highly Susceptible)	Score number 2 (Susceptible)	Score number 1 (Tolerant)	Score number 0 (Resistant)	Disease severity (%)
H2274	30	-	-	-	100
Pintek	24	6	-	-	93.3
Bor pepper	-	21	9	-	85
Sazlıca tomato	15	15	-	-	83.3
Rio Grande	6	24	-	-	73.3
S. pimpinellifolium	-	12	18	-	70

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References

Chiang, K.S., Liu, H.I., and Bock, C.H. 2017. A discussion on disease severity index values. Part I: warning on inherent errors and suggestions to maximise accuracy. Annals of Applied Biology 171(2):139-154

Dombrovsky, A., & Smith, E. (2017). Seed transmission of Tobamoviruses: Aspects of global disease distribution. Adv. Seed Biol, 12, 233-260.

EPPO https://gd.eppo.int/taxon/TOBRFV

FAOSTAT The Food and Agriculture Organization of the United Nations
https://www.fao.org/faostat/en/#data/QCL

Jewehan A, Kiemo FW, Salem N, Tóth Z, Salamon P, Szabó Z. 2022. Isolation and molecular characterization of a tomato brown rugose fruit virus mutant breaking the tobamovirus resistance found in wild Solanum species. Arch. Virol. 167:71559–63

Jewehan, A., Salem, N., Tóth, Z., Salamon, P., & Szabó, Z. (2022). Evaluation of responses to tomato brown rugose fruit virus (ToBRFV) and selection of resistant lines in Solanum habrochaites and Solanum peruvianum germplasm. Journal of general plant pathology, 88(3), 187-196.

Kabas A, Fidan H, Kucukaydin H, Atan HN. 2022. Screening of wild tomato species and interspecific hybrids for resistance/tolerance to tomato brown rugose fruit virus (ToBRFV). Chil. J. Agric. Res. 82:1189–96

Salem, N. M., Abumuslem, M., Turina, M., Samarah, N., Sulaiman, A., Abu-Irmaileh, B., & Ata, Y. (2022). New weed hosts for tomato brown rugose fruit virus in wild Mediterranean vegetation. Plants, 11(17), 2287.

Salem, N. M., Jewehan, A., Aranda, M. A., & Fox, A. (2023). Tomato brown rugose fruit virus pandemic. Annual review of phytopathology, 61(1), 137-164.

Story, E. N., Kopec, R. E., Schwartz, S. J., & Harris, G. K. (2010). An update on the health effects of tomato lycopene. Annual review of food science and technology, 1(1), 189-210.

Tüik https://www.tuik.gov.tr/

Viralzone https://viralzone.expasy.org/51

Viuda-Martos, M., Sanchez-Zapata, E., Sayas-Barberá, E., Sendra, E., Pérez-Álvarez, J. A., & Fernández-López, J. (2014). Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: a review. Critical Reviews in Food Science and Nutrition, 54(8), 1032-1049.

Willcox, J. K., Catignani, G. L., & Lazarus, S. (2003). Tomatoes and cardiovascular health. Critical Reviews in Food Science and Nutrition, 43, 1-18.

Zinger, A., Lapidot, M., Harel, A., Doron-Faigenboim, A., Gelbart, D., & Levin, I. (2021). Identification and mapping of tomato genome loci controlling tolerance and resistance to tomato brown rugose fruit virus. Plants, 10(1), 179.